

AD _____

Award Number: DAMD17-00-1-0174

TITLE: Microsatellite and Chromosomal Instability in Breast Cancer

PRINCIPAL INVESTIGATOR: Svetlana Baranovskaya, Ph.D.

CONTRACTING ORGANIZATION: The Burnham Institute
La Jolla, California 92037

REPORT DATE: July 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20031216 143

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2003	3. REPORT TYPE AND DATES COVERED Annual Summary (1 Jul 2002 - 30 Jun 2003)	
4. TITLE AND SUBTITLE Microsatellite and Chromosomal Instability in Breast Cancer			5. FUNDING NUMBERS DAMD17-00-1-0174	
6. AUTHOR(S) Svetlana Baranovskaya, Ph.D.			8. PERFORMING ORGANIZATION REPORT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Burnham Institute La Jolla, California 92037 E-Mail: svetlana@burnham.org				
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) A set of the microsatellite markers spanning 21 Mbp around the epidermal growth factor receptor (EGFR) gene at 7p12 was used to determine possible chromosomal imbalances in breast tumors. Our study has shown that changes in the EGFR gene copy number is a frequent event in breast cancer and occurs in 22% of breast cancer patients. Eight percent of cases analyzed had an amplification of the EGFR gene containing region. In addition to the gains of the chromosome 7 region, 14% of the cases showed loss of heterozygosity (LOH). The length of the deleted region varied from 2.3 Mbp to the entire chromosome. Therefore, both the amplification and the deletion of the EGFR gene facilitate tumorigenesis in a set of breast tumors that should be reflected in the clinico-pathological parameters of the tumors. This finding has a high importance because epidermal growth factor receptor is a target for some chemotherapy drugs.				
14. SUBJECT TERMS Breast cancer, gene amplification, LOH, EGFR				15. NUMBER OF PAGES 7
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover	1
SF 298	2
Table of Contents	3
Introduction	4
Results	4-5
Discussion and Future Plans	6-7

INTRODUCTION

Two major types of genetic aberrations have been found in breast tumors: chromosomal segment deletions and amplifications, and point mutations. Point mutations often involve microsatellite sequences (microsatellite instability), which were shown to be due to inactivation of the DNA mismatch repair (MMR) machinery.

Although microsatellite instability is common for different cancers, the spectrum of cancer genes that are the targets for microsatellite instability may be cancer site specific. This may be due to the different involvement of chromosomal and microsatellite instability, the two mechanisms that can inactivate tumor suppressor genes. For example, two breast cancer tumor suppressor genes, BRCA1 and BRCA2, contain microsatellite repeats in their coding regions and, thus, may be good targets for microsatellite instability in breast cancer. However, the kinetics of microsatellite instability development and progression in breast cancer has not been evaluated. The relative involvement of microsatellite and chromosomal instability in breast cancer is also not understood.

During the reporting period, we evaluated the degree of chromosomal instability in breast tumors. We checked our panel of breast tumors for chromosomal deletions and amplifications around the epidermal growth factor receptor gene (EGFR) locus, which is located at chromosome 7p12. EGFR is a transmembrane protein, which stimulates cell proliferation while binding to the specific ligands. Overexpression of EGFR has been implicated in malignant transformation in many types of cancers. Our data indicate that the EGFR locus is not only amplified in breast tumors but also is frequently deleted.

RESULTS

We performed microallelotyping analysis of the 7p12 chromosomal region with 14 microsatellite markers, which covered a region of 21 Mbp in length. We also used distal telomeric markers D7S2477, D7S531, D7S2423 and D7S550 to determine if the whole chromosomal arm was gained or deleted in tumors. We employed multiplexed PCR to unambiguously distinguish chromosomal gains versus chromosomal losses (Fig. 1). The results of this analysis are summarized in Fig. 2.

This allelotyping analysis revealed two amplicons, 1,318 kbp and 2,202 kbp in length. The overlapping part of the two amplicons spanned 846 kbp and contained the EGFR gene. Two more cases (120, 108) showed allelic gain at several consecutive markers (including telomeric), which indicated a copy number gain of 7p for the first case and the entire chromosome 7 for the second one.

In addition to the gains of the 7p12 region, 14% of breast tumors demonstrated loss of heterozygosity (LOH). The lengths of deleted chromosomal segments ranged from 2.3Mbp (for example, case 112) to the entire chromosome (for example, case 26 and 91). The smallest common region of these chromosomal losses contained the EGFR gene.

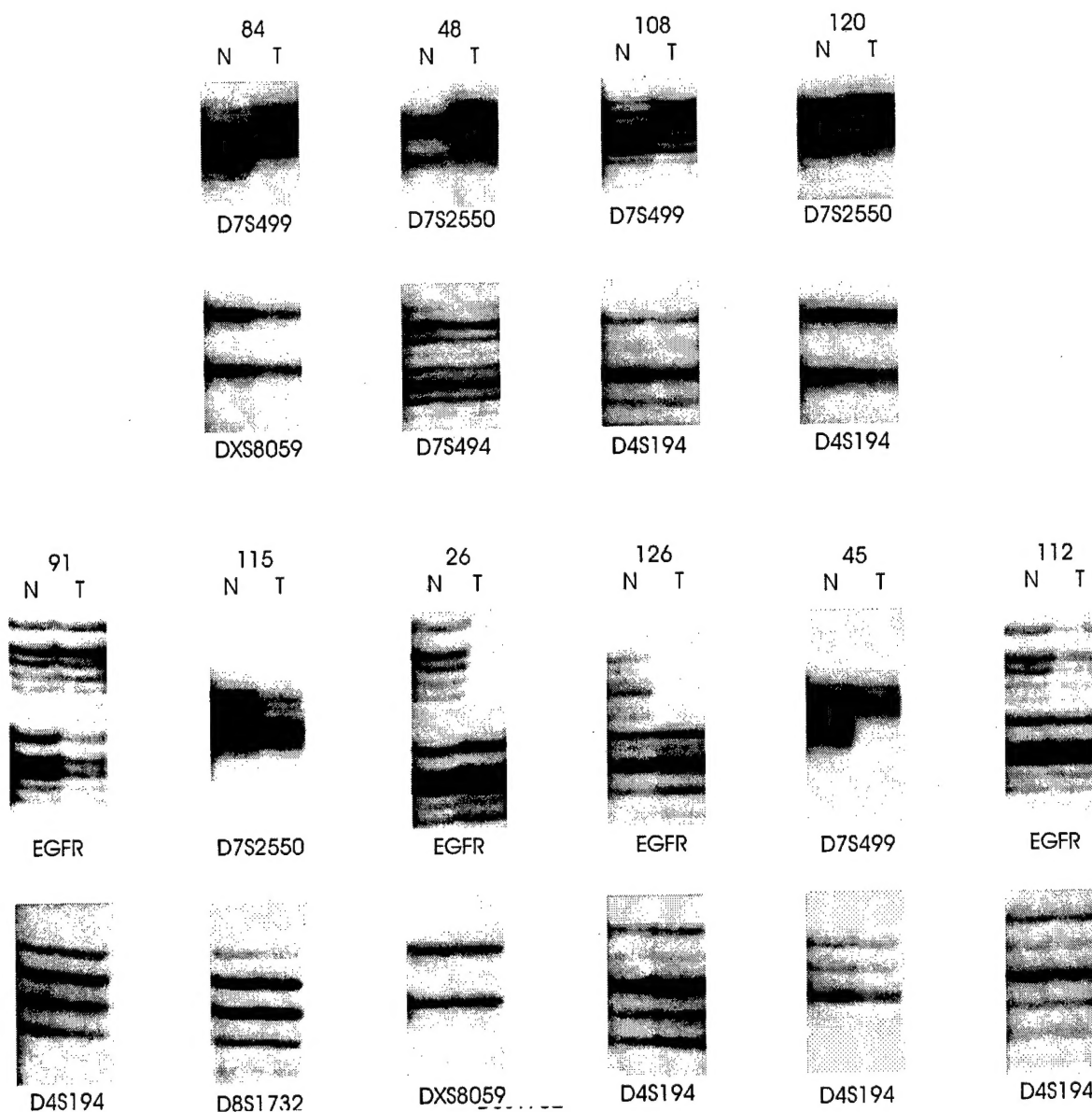


Fig. 1. Allelotyping of the breast tumors by multiplexed PCR. Top panel: microsatellite markers to be examined for gain/LOH. Bottom panel: microsatellite markers without allelic imbalances used as a reference (control). Names of the microsatellite markers analyzed are shown under the corresponding pictures. N, normal tissue; T, tumor tissue. Gain at the D7S499 in cases 84 and 108; at the D72550 loci in cases 48 and 120. LOH at the EGFR loci in cases 91, 26, 126 and 112; at the D72550 in case 115; at the D7499 in case 45.

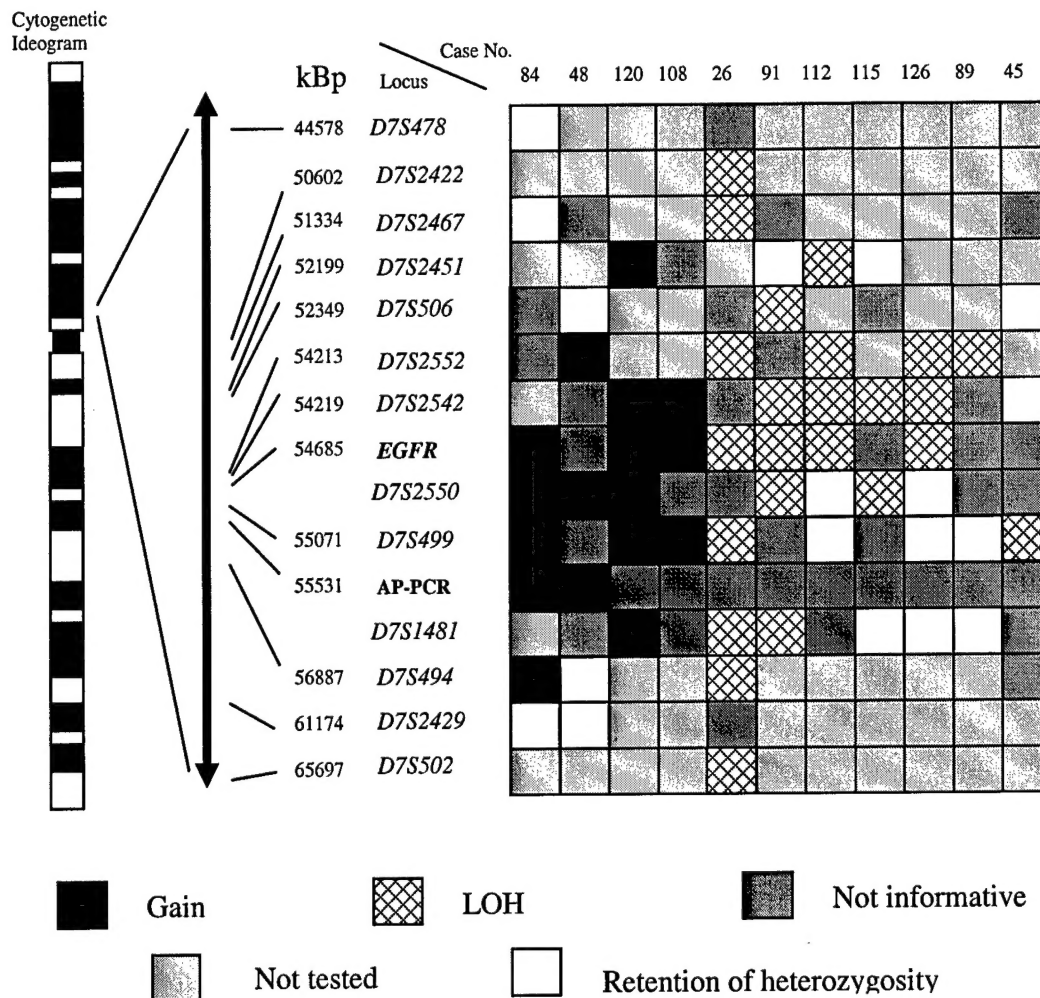


Fig. 2. Maps of deletions and gains at 7p12 chromosomal region in breast tumors.

DISCUSSION AND FUTURE PLANS

Frequent LOH at a particular chromosomal region is generally thought to reflect the existence of a tumor suppressor gene within the lost region. A negative role of the EGFR gene in colonic tumorigenesis has been illustrated previously. It is possible that EGFR may play a negative role in tumorigenesis of breast cancer in some cases and a positive in another. Our prediction is that this should be reflected in clinico-pathological characteristics of tumors and, possibly, in the response to different anticancer therapies. This could be especially important because overexpression of EGFR is a target for some chemotherapy drugs.

Next year, we plan to screen our panel of breast tumors for the presence of microsatellite instability. So far, the microsatellites used for allelotyping studies have not detected

microsatellite instability. We plan to use PCR primers for polyA sequences within BRCA1 and BRCA2 genes to detect possible deletion/insertion mutations, which are characteristic for microsatellite instability phenotype.

Publications:

Baranovskaya S., Malkhosyan SR. Frequent copy number alterations of chromosome 7p12 region in breast carcinomas (in preparation).